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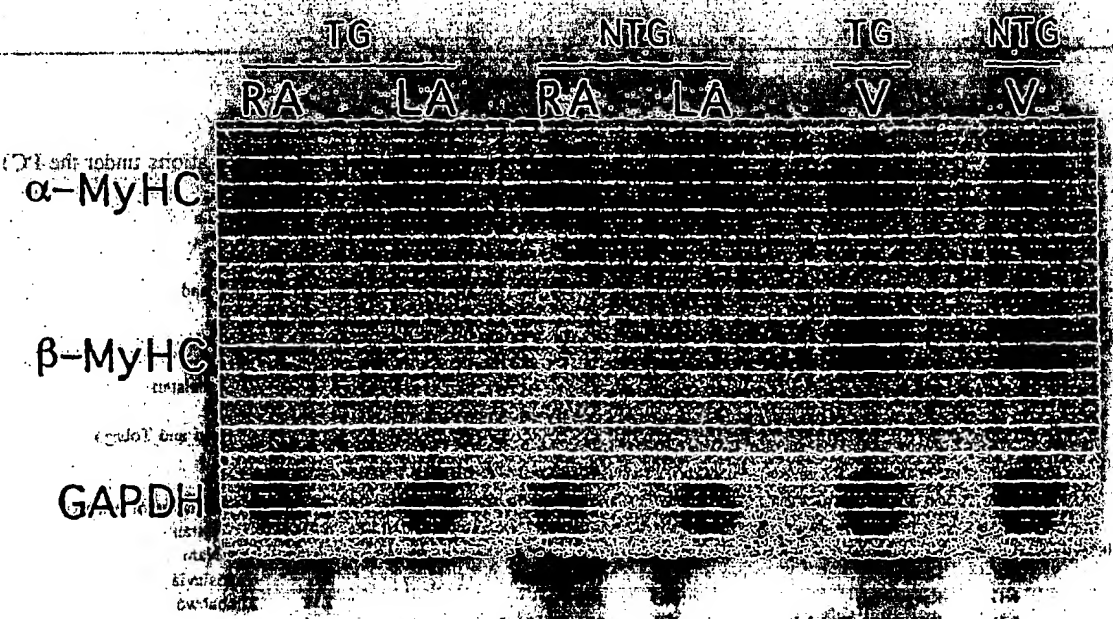

(57) Abstract

A murine α and β murine myosin heavy chain (MyHC) promoter is used in gene transfer, gene therapy, and production of transgenics. In larger animals the promoter is expressed in only striated muscle. Therefore, the promoter can be used for efficient, high levels of expression of a gene of interest specifically in striated muscle tissue. This makes it perfect for use in gene therapy of muscle-related diseases such as: Duschenne Muscular Dystrophy (DMD) and even systemic diseases, particularly inflammatory diseases.

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USE OF MURINE MYOSIN HEAVY CHAIN PROMOTERS FOR GENE THERAPY AND PRODUCTION OF TRANSGENICS

FIELD OF THE INVENTION

This invention relates generally to the use of exogenous promoters for tissue specific exogenous gene expression. More specifically, the present invention relates to the use of trans-species striated muscle specific promoters, such as the murine alpha and beta myosin heavy chain promoters, which can be used for tissue specific exogenous gene expression in gene therapy, gene transfer, and for the production of transgenic animals.

BACKGROUND OF THE INVENTION

The past few years have witnessed tremendous growth in the field of gene replacement therapy for treating mammalian diseases. Gene therapy has been considered for numerous inherited and acquired disorders. Studies have utilized a wide range of vectors including retroviral, adenoviral, plasmid and naked DNA vectors. However, the technology that currently exists in the fields of exogenous gene transfer gene therapy suffers a number of limitations that restrict the availability of this technology for clinical uses. As discussed below, one significant limitation is the lack of specificity that is evident in most of the vector systems used in present day gene transfer protocols. As with any therapeutic treatment, specificity is a needed and desired characteristic to minimize the risk of adverse effects.

The process of gene transfer generally involves the use of a vector system. A vector system is the means used to carry the exogenous genetic material into the subject for later expression. One of the more efficient vector systems involves the use of recombinant retroviral vectors. Retroviruses have tremendous potential for use as delivery or vector systems because the genetic content of the retroviral genome incorporates itself into the infected cell. By doing so, a recombinantly generated retrovirus that carries an exogenous gene of interest can be used to introduce that gene into the genome of a target cell. This incorporation means that as the target cell gives rise to progeny, each of the daughter cells will carry the exogenous gene of interest. Vectors that do not incorporate into the genome provide only transient expression of the exogenous gene. Unfortunately, retroviruses require their target cells to be dividing for incorporation to occur. Thus, the overall population of cells that are available for retroviral infection may, depending upon the target tissue, be limited.

Other viral vector systems have been explored as potential vector systems for gene transfer. Adenoviral vectors have shown great promise in the area. These viruses can be grown in large quantities and can infect nondividing cells. However, the adenoviral systems suffer from a limitation common to viral vectors, the adenoviral tropism limits the types cells into which the adenoviral genome can be introduced.

Another types of non-vector delivery involves the use of naked DNA. Naked DNA can be in the form of a plasmid, viral DNA, or cDNA. Naked DNA is taken up by, and believed to be expressed at high levels in hepatocytes and lower levels in muscle. Other non-viral methods include liposomal transfer.

A problem common to all vector systems is their cell or tissue specificity, or tropism. Some vectors, such as viruses, may have very specific cell types that they infect. For example, the tropism of the human immunodeficiency virus (HIV) is limited largely by the fact that the virus binds to the CD4 protein presented on T cells. Other vector systems have a very low cell, tissue, or organ specificity. In view of these limitations, a gene transfer system that provided for the tissue specific expression of a gene of interest would clearly result in fewer side effects and more efficient treatment.

Tissue targeting can be accomplished in a number of ways. Each technique has its own advantages and disadvantages. A simple technique for tissue tropism is accomplished by applying the therapeutic gene or vector only to the target tissue. Another technique involves using the natural tropism of the viral vector. This tropism can be manipulated producing a viral vector that is specific for a different tissue. Another technique involves making the therapeutic gene transcriptionally specific to the targeted cell or tissue. In other words, the therepeutic gene will only be expressed in the targeted tissue.

One example of applying the needed therapy only to the targeted area is the following. Clinical trials have concentrated on using adenoviral vectors for the treatment of cystic fibrosis by using inhalation directly into the lungs to target the adenoviral vector to the cells which need the therapy. Because adenovirus naturally infects respiratory cells, this takes advantage of the natural tropism of the virus. However, this technique is limited to the use of vectors which naturally infect only the targeted cells. Because suitable viral vectors are limited, this severely limits the variety of tissues which can be targeted.

Another example involves direct injection of naked DNA into muscle tissue in the form of cDNAs, plasmids, or even viral vectors. It can be imagined that only certain diseases would be amenable to this type of localized application, thus limiting the diseases which can be targeted.

Another approach involves the used of targeted ligands which can be genetically introduced into a viral protein. This approach involves extensive manipulation of viral genomes and may or may not be efficient. In addition, it can only be used for viral vectors.

All of the above methods have the additionally drawback of non-specificity. They will be expressed wherever they are taken up, not just the targeted cell or tissue. This can lead to side effects and a general lack of control over the system.

Skeletal Muscle as a Target Tissue

Muscle can be separated into two types, striated or non-striated. Striated muscle includes cardiac and skeletal muscle. This is because the two types of muscle have similar sarcomeric organization. The non-striated muscle is the smooth muscle of most organs. We are interested in the study of striated muscle.

Decades of research in the etiology and treatment of skeletal muscle diseases has led to the following conclusions: a) traditional therapeutic approaches to the treatment of these disorders have been, at best, marginally effective, and b) novel approaches utilizing gene therapy are a possible answer to treating and/or curing these diseases. Current and proposed gene therapeutic approaches often depend upon non-specific transcriptional control elements to drive high levels of the therapeutic gene. The technology that currently exists is limited to viral promoters which are strong but non-specific. They also have the added problems that they are both inefficient and have the potential for driving transgenic expression in undesired directions. A number of studies suggest that the use of skeletal muscle as a target tissue for gene therapy shows great promise for the treatment of muscle-based diseases as well as for the treatment of many systemic diseases, particularly inflammatory diseases. However, an efficient muscle-specific system for use in all vector types is yet to be developed.

Several stratagies have been developed to introduce foreign genes into diseased muscles, including myoblast transfer, direct injection of plasmids or DNA-liposome complexes, and infection with modified viuses. Related strategies using antisense sequences or ribozymes, have been devised to modify gene expression in diseased cells.

Douglas, J.T. et al (1997, Neuromuscular Disorders, 7, pages 284-98) developed a strategy to modify the tropism of adenoviral vectors to produce muscle specific delivery. Normally adenovirus infects epithelial cells of the respiratory tract. Douglas, et al. introduced targeting ligands into the adenovirus fibre, which mediates the binding of the viral protein to the primary cellular receptor. This chimeric receptor changed the tropism of

the virus to muscle cells. However, this type of modification requires extensive manipulation of viral genes and would only be useful for viral vectors.

An alternative technique for producing a muscle-specific vector involves direct injection of a Herpes simplex virus type 1 (HSV-1) vector into the muscle (Huard, J. et al, 1997, Neuromuscular Disorders 7, 299-313). The viral genome is large and can accommodate large non-viral genes. However, there are a number of impediments to using this system. Namely, viral cytotoxicity and the differential transducibility with HSV-1 mutants throughout the development of muscle fibers. In addition, it requires the direct injection into the muscle to produce specificity.

Direct injection of naked DNA or adenovirus-based vectors into the muscle has the same drawbacks. Namely, inefficiency of application, requiring injection into the muscles where it is needed.

Duchenne Muscular Dystrophy

Production of a muscle-specific vector could be useful in many ways. The obvious value is in treating muscle-specific inherited and acquired diseases. However, there is also some reason to believe that a muscle specific delivery could be useful for treatment of a number of systemic diseases and more specifically, inflammatory diseases. However, the disease prototype of muscle-specific gene therapy is Duchenne Muscular Dystropy (DMD). Experiments in dystrophin gene transgenic mice have supported the concept of treating Duchenne Muscular Dystrophy (DMD) with gene therapy. These experiments demonstrated that regional expression of recombinant dystrophin in dystrophic muscle leads to regional restoration of normal muscle morphology. It also suggests that dystrophin mini-genes driven by muscle specific regulatory elements are probaby more effective than the full-length dystrophin gene. Inui, et al (1996, Brain & Development 18, pages 357-61) introduced dystrophin cDNAs into skeletal muscle fibers of dystrophin-deficient mice (mdx) through direct DNA injection into plasmid expression vectors, and by replication-defective recombinant retrovirus or adenovirus vectors. Less than 10% of adult mdx fibers of the plasmid and retrovirus injected muscle expressed dystrophin. This very low efficiency provides some hope for such treatment, however, it is widely believed that specific tropism or gene transcriptional activity is vital for treatment of DMD.

Other Uses for a Muscle-Specific Vector System

There are a number of other uses for a muscle-specific vector system particularly in research-related activities. One use is for producing transgenics which express various muscle-specific genes or turn off others. These transgenics would be useful in

understanding the role of these genes in muscle development. In addition, animal models of muscle-specific and cardiac diseases could be developed for use in researching therapeutics. Lastly, muscle-specific vector systems could be used *in vitro* to more efficiently transfer genes into muscle-related cell types.

SUMMARY OF THE INVENTION

The invention is a selectively modified myosin promoter which drives high levels of protein expression very efficiently in muscle tissue such that they offer the ability to direct in a specific manner to striated muscle, very high and efficient delivery of transgene expression. The α and β myosin heavy chain promoter (MyHC) will drive expression of gene therapeutics at high levels in striated muscle. The promoter is inactive in non-muscle tissue or in smooth muscle, lending the desired degree of specificity to the biological delivery systems. This has previously been unobtainable for a promoter that is able to drive very high levels of transgene expression in striated muscle types of large mammalian species. Therefore, there has long been a need for a tissue specific method of gene transfer. The promoter of the invention fills this need.

One object of the invention is to provide a vector for expressing an exogenous DNA in a muscle specific manner which is made up of a murine myosin heavy chain promoter or variants thereof which are capable of expressing in a muscle-specific manner which is expressing an exogenous DNA. The vector preferably uses the α or β murine myosin heavy chain promoter. It is prefered that the muscle specificity is to striated muscle. More preferably, the promoter and exogenous DNA are contained in a delivery system, preferably, a virus, plasmid, or liposomes. The exogenous DNA is preferably a muscle-specific gene, heart-specific gene, anti-inflammatory gene, antisense DNA, ribozyme, or systemic disease gene. The muscle-specific gene is preferably, the Dystrophin gene, the Dystrophin mini-gene, the Utrophin gene, or variants thereof, dystroglycans, emerin and tropomyosin. The systemic disease genes are preferably Factor IX or decorin.

A further object of the invention is to provide a method for expressing exogenous DNA in a muscle-specific cell, organism, or tissue having the steps of a) selecting an exogenous gene b) genetically attaching it to the murine myosin heavy chain promoter or variants thereof producing a promoter construct, such that the promoter controls expression of the exogenous DNA, and c) delivering the promoter construct to a cell, organism or tissue

using a delivery system. Preferably, the promoter is the α or β murine myosin heavy chain promoter, preferably confering muscle specificity to striated muscle. Preferably, the exogenous DNA is a muscle-specific gene, heart-specific gene, anti-inflammatory gene, antisense DNA, ribozyme, or systemic disease gene. More preferably, the muscle-specific gene is the Dystrophin gene, the Dystrophin mini-gene, the Utrophin gene, or variants thereof, dystroglycans, emerin, and tropomyosin. More preferably, the systemic disease genes are Factor IX or decorin. Preferably, the delivery system is a viral vector, a plasmid, a liposome, or Naked DNA. The exogenous DNA can be delivered to said cell, organism, or tissue *in vitro* or *in vivo*.

A further object of the invention is a method for producing a transgenic with muscle-specific expression of an exogenous DNA having the steps of a) selecting an exogenous gene or DNA, b) producing a functional promoter by attaching it to the murine myosin heavy chain promoter or variants thereof producing a promoter construct, and c) delivering the promoter construct to an egg, blastocyst or zygote. The promoter is preferably the α or β murine myosin heavy chain promoter. It is prefered that the muscle specificity is to striated muscle. Preferably, the exogenous gene or DNA is a muscle-specific gene, heart-specific gene, antisense DNA, and variants thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1C. Transgene Expression in the Heart.

Figures 2A-2B. CAT expression is homogeneous throughout muscle. A. Alpha CAT line 290 papillary muscle. B. Nontransgenic papillary muscle.

Figures 3A-3B. CAT expression in skeletal muscle. A. Alpha CAT line 286. B. Alpha CAT line 290.

Figure 4. Endogenous expression of α-MyHC and β-MyHC in transgenic rabbits in Alpha CAT line 290, the highest expressing line. α-MyHC = alpha myosin heavy chain, β-MyHC = beta myosin heavy chain; GAPDH = glyceraldehyde phosphatase dehydrogenase; RA = right atrium; LA = left atrium; V = ventricle, TG = transgenic; NTG = nontransgenic.

Figures 5A-5B. CAT expression driven by the mouse β–MyHC promoter in transgenic rabbits. A. Beta CAT line 492 cardiac expression. B. Beta CAT line 492 muscle expression.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the use of modified and unmodified myosin promoters from an exogenous species that drive high levels of protein expression in striated muscle tissue. The promoters of the present invention are substantially inactive in non-muscle tissue or in smooth muscle. This tissue specific activity provides the desired degree of specificity to the various gene delivery systems. This specificity has previously been unobtainable for a promoter that is able to drive very high levels of transgene expression in striated-muscle types of large mammalian species. Therefore, this technology will fill a long felt need for a strong, striated-muscle specific promoter suitable for biologic delivery.

The exogenous promoters described by the present invention are highly useful for efficacious and selective gene transfer protocols. In one embodiment, the promoters of the present invention are used as part of a therapeutic modality for the treatment of inherited skeletal muscle disorders. In addition, these promoters can be used in any gene delivery system where a striated muscle limited expression of the gene of interest is desired. Additionally, since striated muscle is a secretory tissue, the general approach holds open the possibility of systemic delivery if desired.

Vector Delivery Systems

The introduction of the exogenous promoters taught by the present invention can be accomplished using a number of vector or delivery systems. Examples of such vector systems include but are not limited to viral vectors, plasmid DNA, cDNA constructs, liposomes, naked DNA constructs, and other delivery systems known to those of skill in the art.

Examples of viral delivery systems are retroviral or lentiviral. These are very efficient but have the disadvantage that current vectors only incorporate into proliferating cells. Current work on modifying the vectors so that they incorporate into nonproliferating cells is showing promise. Another example of a suitable viral vector system is the adenoviral system. Adenoviral vectors will incorporate into nonproliferating cells. However, adenoviral DNA does not integrate into host nuclei, but nonetheless it persists in postmitotic myofibers for up to 6 months. Herpesviral and other viral vectors are also being

Plasmid and naked DNA require direct DNA injection. Muscle appears to have a remarkably high capacity to internalize DNA and plasmids, and to express foreign proteins. One advantage is that this type of therapy does not possess the biohazard that many of the current viral vectors have.

Myosin Heavy Chain Promoters

The present invention contemplates the use of myosin heavy chain promoters from one species to drive striated muscle specific gene expression in a different species. A murine myosin heavy chain promoter is a promoter which is operably linked to murine myosin heavy chain. In one embodiment of the present invention, myosin heavy chain promoters of murine origin are used to drive striated muscle specific expression in a rabbit model. However, use of these promoters in any non-murine host is contemplated.

In both lower organisms and in mammals, an important component of the contractile apparatus is the myosin heavy chain (MyHC). The myosin heavy chain protein is encoded by a large gene family. The members of this multigene family are differentially expressed in a developmental stage- and muscle type-specific manner. In mammalian cardiac muscle, two of the gene family's members, termed α-MyHC and β-MyHC and are thought to play a critical role in determining the speed of contraction. Other myosin heavy chain promoters are specifically expressed in skeletal muscle and even more specifically in fast skeletal muscle (fast skeletal myosin heavy chain promoter).

In adult murine atrium, α-MyHC is expressed constitutively. However, in the embryonic/foetal ventricle, the β-gene is predominantly expressed. At or around birth there is an antithetic switch of β to α in the ventricle such that the V3 isoform is gradually replaced by the V1 protein. Thus, >95% of the MyHC transcripts in the mature ventricle are transcribed from α-MyHC with only trace amounts of the β-gene-encoded RNA being present.

Previous studies have defined what parts of the promoter are necessary for high levels of transcription. The murine myosin heavy chain promoter contains thyroid response elements (TREs) identified in the proximal promoter region. Expression from the TREs is controlled by thyroid hormone (TH). Direct injections of DNA into the myocardium have shown that 612 bp of the gene's upstream region is sufficient to confer TH modulation to a reporter gene construct *in vivo* (Kitsis et al., 1991 P.N.A.S. Vol 88; pp. 4138-42). Site-directed mutagenesis of the α-MyHC promoter in a transgenic analysis has been used to define those elements responsible for high levels of transcription *in vivo*. Because of the similarity between the promoters, these studies can be applied to other myosin heavy

promoter. TRE_1 and TRE_2 are located at -129 to -149 and -102 to -120, respectively, on the α-MyHC promoter. Although the elements' ablation had differential effects on transgene expression, neither single mutation abolished transgene expression completely, however, each TRE alone only had about 10% of normal activity. Mutating both elements resulted in a complete inactivation of the transgene in both ventricles and atria under conditions with no thyroid hormone. In hyper-thyroid conditions, expression can still be detected. Therefore, although TRE_1 and TRE_2 elements are critical elements for high levels of α-MyHC transcription *in vivo*, other promoter sites can mediate at least some degree of transcriptional activation. Both elements are needed for the high level of gene expression as well as developmental regulation. This suggests that other parts of the promoter would not be necessary for this high level of expression.

Exogenous DNA or The Gene of Interest

The gene of interest is any gene which is capable of being expressed in the system. The gene may be of interest for experimental reasons or for treatment of a disease. Preferably, expression of the gene product would alleviate a disease. Examples include diseases due to loss of a functional gene product, such as Duchenne Muscular Dystrophy (DMD) which has lost the gene dystrophin, limb-girdle dystrophy which has lost dystroglycans, Emery-Dreifuss disease which has lost emerin, and nemaline rod myopathy which has lost tropomyosin.

Alternatively, the exogenous DNA is a gene product which would alleviate diseases due to mutation or aberrent expression of a gene product or virus. These could be treated with antisense DNA or ribozymes. Alternatively, the promoter is used to produce a gene of interest which acts as a vaccine.

The method of the present invention can also be used to generate transgenic animals. In this embodiment, a gene transfer vector containing a gene of interest and exogenous promoters is introduced into a target cell line. Those cells are then used to generate an entire subject animal in which the gene of interest has been incorporated.

Rabbit transgenic

Successful transgenic investigations begins with the choice of a promoter. Initial transgenic investigations in the mouse made use of non-tissue specific promoters to drive expression of the transgene of interest. In the mouse, the α-MyHC promoter is capable of driving high levels of transgene expression in a developmental stage- and cardiac compartment- specific fashion, with promoter-driven expression corresponding to the endogenous expression pattern of α-MyHC. Additionally, the expression level is generally

proportional to transgene copy number. The mouse β-MyHC promoter also displays developmental stage- and compartment specific activity and in the adult mouse expresses in the cardiac ventricle and the slow soleus muscle.

Previous work demonstrated that the proximal rabbit α-MyHC and β-MyHC promoters share approximately 85% homology with the mouse promoters in the most proximal 600 base pairs. Since the proximal promoter is responsible for cardiac specificity and this region is essentially conserved between mouse and rabbit, we hypothesized that, as in murine transgenics, the mouse promoters might be useful in remodeling the protein complement of the rabbit heart. Additionally, heterologous promoters have been used successfully to create transgenic animals (including transgenic rabbits). It was found that heterologous use of the murine myosin heavy chain promoters does result in the efficient transcription of a target transgene in the heart of the rabbit. However, surprisingly, it also resulted in efficient transcription in the striated muscles.

Use of muscle-specific promoters in Gene transfer

A specific promoter which is capable of a very high level of expression in striated muscle has a clear use in *in vitro* and *in vivo* studies. Vectors for expressing exogenous genes in tissue culture which can express at high levels and only in specific tissues are needed for experimental systems. For example, the promoter of the present invention is very useful for expressing exogenous genes in muscle-related cell lines such as, myoblasts, myotubes, myogenic cell lines, transformed cell lines and possibly muscle-related cancers such a rhabdomyosarcoma, etc. In experimental studies exogenous genes are expressed for a variety of reasons. For example genes are expressed in undifferentiated cell lines to determine if they are involved in differentiation of the cells toward the muscle phenotype, antisense DNA is expressed in cell lines to determine the effect of a newly discovered gene product, developmental genes are expressed to determine the effect on a differentiated muscle cell, etc.

Utrophin (Dystrophin) expression vector

The main goal of gene therapy for Duchenne muscular dystrophy (DMD) is to restore dystrophin (or a related protein) into as many muscle cells as necessary to be therapeutic. Experiments outlined in the Background have supported the concept of treating DMD in this way by demonstrating that regional expression of recombinant dystrophin in dystrophic muscle leads to regional restoration of normal muscle morphology. In addition, dystrophic mini-genes driven by muscle specific regulatory elements are more effective than the full-length dystrophin gene. Lastly, the related gene utrophin has been used to express

DMD in mdx mice. The α-MyHC and β-MyHC promoters are prime examples of such muscle-specific regulatory elements.

Non-muscle specific disease expression vector for gene therapy

Alternatively, the muscles serve as an excellent site for the production of genetically engineered proteins that may be therapeutic for conditions other than primary myopathies. For example, species or trans-species Factor IX for hemophilia, decorin or antisense TGF-β for kidney fibrosis, the specific allergen for allergic reaction, and a variety of proteins for immunorejection. In addition, vaccines can be produced or antisense and ribozymes.

Heart function in non-murine transgenic animals

The study of the cardiovascular system has benefited tremendously from the use of genetically altered animals, specifically gene-targeted and transgenic mice. Virtually all facets of the cardiovascular system, including cardiac development, the conduction system, the coronary vasculature, the adrenergic system, and the components of the sarcomere have been explored using these technologies. Augmentation of *in vitro* preparations with *in vivo* models has been invaluable in providing integrative data regarding physiological and pathological states in the heart, such as cardiac hypertrophy and dilation. These animals provide the potential reagents to explore complex signaling pathways mediating the transitions from normal cardiac function through compensated cardiac dysfunction to heart failure. Cardiovascular disease remains the leading cause of death in developed countries. There is an urgent need for valid experimental systems to study the pathological progression of cardiovascular disease at all levels (molecular to whole animal) in order to dissect the pathological basis of disease and facilitate the discovery of novel therapeutic agents.

Because of the ease with which the genome may be manipulated and the relatively low cost of maintaining large colonies, most molecular investigations of the cardiovascular system to date have used mice, although in some cases, transgenic rats have been studied. However, the mouse and rat do not accurately reflect potentially crucial facets of human cardiovascular physiology. Indeed, a number of experimental models aimed at duplicating human pathological states by expressing correlative genetic mutations of human genes in small mammals have failed to accurately reproduce the human phenotype. This should not be surprising since the murine heart differs from the human in several very significant features. From a functional standpoint, the mouse heart beats 600 - 700 times per minute and supplies cardiac output for a body mass of 20-40 grams. In contrast, the adult human heart at rest beats 50 - 100 times per minute, supplying cardiac output to a body mass of 50-

95 kilograms. The divergence in cardiac demand is reflected at the molecular level. For example, the most abundant transcripts of the cardiac sarcomere, the myosin heavy chains (MyHCs), are present as two isoforms: the "fast" alpha MyHC isoform, (α–MyHC) and the "slow" beta MyHC (β–MyHC) designated "fast" and "slow" in reference to the relative rates of ATPase activity inherent to these enzymatically active proteins. The normal adult mouse ventricle expresses only the "fast" (α–MyHC) isoform, while the normal human ventricle expresses a mixture of the "slow" β–MyHC and fast" α–MyHC, with the β–MyHC isoform predominating in the healthy adult state.

In addition to the molecular and physiological differences between the mouse and human heart, the challenges and limitations posed by physiological analyses of small mammals are important considerations. There are a number of invasive techniques available to study the mouse cardiovascular function including the isolated heart (Langendorff and working heart preparations), pressure-dimension loops, in situ open chest assessment of +/- dP/dT, pressure-volume loops, and open chest electrophysiology studies. These techniques, while providing detailed physiological assessments are limited to a single experiment per animal since the mouse subject does not survive the procedure. Molecular resonance imaging (MRI) has been used to assess fetal mouse cardiac development. Cardiac function analyses can performed *in vivo* at only very specialized centers because of the technical problems posed by the rapid heart and respiratory rates of the mouse. Transthoracic echocardiography has been widely used as a method to repeatedly assess cardiac function in mice but the quality of data obtained is highly user dependent and complex, load-independent measurements cannot be reliably obtained. All told, despite a great deal of effort over the last eight years, only a limited number of laboratories are capable of performing these assays, leaving the bulk of the research community with serious accessibility issues. Thus reproducible data remain limited.

It would thus be beneficial to move selected models into larger mammalian transgenic animals other than the rabbit. The rabbit was a good choice to start with because the gestation period is relatively short (30 days) and sexual maturity occurs relatively quickly (20 - 24 weeks). The rabbit is a very useful model for studying aspects of human heart disease and transgenics can be made in a relatively straightforward manner. At the molecular level, rabbit atria express the α–MyHC isoform at all stages of development while the ventricles express both the α– and β–MyHC isoforms, with the β–MyHC isoform dominating in adulthood. This MyHC expression pattern is essentially identical to that of

between mouse and human. At 200-300 beats/minute, the rabbit has a significantly slower heart rate than the mouse and approaches that of a human neonate. These physiological parameters make the rabbit an attractive model for cardiovascular research since the modalities available for clinical evaluation of human cardiac function can be more readily adapted for the rabbit heart.

Muscle function in non-murine transgenics

The promoter of the present invention will be used to produce non-murine transgenics for the purpose of understanding the role a protein plays in muscle development and disease. Much can be learned by over-expressing the protein product or a mutated version, or by producing an antisense DNA. The effect will be limited to striated muscle.

Further features and advantages of the present invention will become apparent to those of skill in the art in view of the detailed description of the invention which follows when considered together with the attached drawings and claims.

EXAMPLE 1

Murine Myosin Heavy Chain Promoter Constructs

Example 1 describes the construction of a murine myosin heavy chain promoter driven gene expression cassette. The full-length mouse α-MyHC and β-MyHC promoters has been extensively characterized using chloramphenicol acetyl transferase (CAT) as the reporter gene (Rindt, H. et al. 1995. Transgenic Research 4, 397-405). Briefly, all critical transcriptional components are conserved upstream of the cDNA insertion site. This includes exon-intron splicing junctions and a strong translational start signal. Downstream are three stop codons in all possible frames and a polyadenylation site.

These α-MyHC/CATand β-MyHC/CAT constructs (α/CAT and β/CAT, respectively) are free of cloning artifacts and thus were used in the generation of transgenic rabbits without modification. The promoter sequence and CAT reporter gene were excised from the plasmid by Not I digest and the desired fragment isolated by gel purification and subsequent dialysis against TE (10 mM Tris, pH 7.0, 0.1mM EDTA).

For other types of experiments, the promoter itself is excised and subcloned into the vector, virus, plasmid, cDNA, or other delivery mechanism of choice. The promoter will then be used to express exogenous DNA.

The murine promoter described in Example 1 was used to produce a rabbit transgenic as shown in Examples 2-9

EXAMPLE 2

Generation of transgenic rabbits

The full-length mouse α–MyHC and β–MyHC promoters were used in the generation of transgenic rabbits without modification. The promoter sequence and CAT reporter gene were excised from the plasmid by Not I digest and the desired fragment isolated by gel purification and subsequent dialysis against TE (10 mM Tris, pH 7.0, 0.1mM EDTA).

The standard injection protocol for transgenic mice was modified to a four-day procedure in the rabbit to account for timing differences in ovulation and fertilization. All experiments were performed with New Zealand White rabbits under a protocol approved by the Animal Care Committee. The oocyte donor doe was super-ovulated on day one of the protocol with 150 units pregnant mare serum gonadotropin (PMSG) delivered subcutaneously under the scruff of the neck. On day three, the donor doe was mated with a non-transgenic buck. Additionally, both the donor and recipient does received 150 units of human choriogonadotropin (HCG) administered in an ear vein. On day four, the eggs were harvested from the donor doe and the pronucleus of viable eggs injected with purified DNA. The injected eggs were then transplanted into the fallopian tube of the pseudopregnant donor. The recipient doe was moved to a nesting cage two to three days prior to the expected delivery date. Transgenic offspring were identified by PCR (using CAT specific primers) and genomic Southern (with 32P-labelled CAT cDNA as the probe). The founder rabbits were aged to five months (females) or six months (males) before attempting to breed for F1 offspring. F1 and/or F2 offspring were used for all subsequent analyses. Table 1 summarizes our experience in founder generation.

Table 1. Transgenic rabbit generation

Eggs recovered	1403
Viable eggs injected	1000
Potential founder (FO) kittens	120
Still born	33
Liveborn	87
Confirmed FO animals	11
Integration pattern of the transgene	
Mosaic	9

Germline	2
Number of lines with detectable transgene expression	4

Numbers include our experience with both the mouse α-MyHC/CAT(α/CAT) and the mouse β-MyHC/CAT(β/CAT) constructs.

Diploid copy number was determined with DNA dot blots using a ^{32}P-labelled CAT cDNA probe. The blots were placed on a phosphor screen, the image scanned with a STORM 760 machine and the results analyzed using Image Quant Mac1.2 (Molecular Dynamics, Sunnyvale, CA). The overall success rate is shown in Table 1. From 1000 reimplanted embryos, 87 liveborn rabbits were obtained, of which 11 were transgenic. These results gave an overall efficiency of approximately 1%, (approximately 13% for live born rabbits). The success rate for the generation of transgenic mice was approximately 25%. Thus, the success rate for rabbits was less than the success with mice, but similar to what has been reported by others.

It was noted that the degree of mosaicism in our founder rabbits exceeded the mosaic rate in mice, but was not significantly different from published experiences in other laboratories. The increased incidence of transgenic mosaicism in rabbits is likely due to differences between mice and rabbits in the timing of DNA integration and repair.

A total of seven α/CAT founders were generated, of which two transmitted the transgene to the F1 generation in a pattern consistent with germline integration of the transgene (i.e., approximately one-half of each F1 litter was transgenic). In the lines with few or no transgenic offspring, an embryonic lethal phenotype is formally possible, but unlikely given the extremely high levels of CAT protein that some lines demonstrated without any apparent pathology. For the analyses reported here, three transgenic lines were used.

EXAMPLE 3

Cardiac expression patterns of α and β MyHC in the transgenic rabbit

Non-transgenic rabbits ages 3-5 days, 8-12 days, 4-6 weeks, 8-12 weeks, and >16 weeks were sedated with intramuscular ketamine then euthanized with intravenous pentobarbital. After the heart was quickly isolated, atrial and ventricular tissue was dissected and frozen in liquid nitrogen. Total RNA was extracted with TriReagent (Molecular Research Center, Inc, Cincinnati, OH). RNA dot blots were performed on nitrocellulose with atrial and ventricular total RNA using one microgram of total RNA per

dot. All hybridization steps were performed in a 55°C water bath. The blots were pre-wetted with 0.2X SSC for 10 minutes, then prehybridized for one hour in Denhardt's solution with 5X SSC. Transcript specific oligonucleotides for rabbit α–MyHC, β–MyHC, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were labeled with ^{32}P-ATP using T4 polynucleotide kinase (Gibco BRL, Gaithersburg, MD) and added to the prehybridization solution to a final concentration of 1 x 106 dpm/ml. Hybridization proceeded for five hours. After three ten minute washes with 0.7X SSC/1% SDS, the blots were placed on a phosphor screen overnight then scanned and analyzed as described above.

Expression of the transgene was analyzed by CAT enzyme-linked immunoabsorption assay (ELISA). Transgenic rabbits were sacrificed at 3-5 days, 8-12 days, 4-6 weeks, 8-12 weeks, and > 16 weeks as described above. Tissue samples were dissected from multiple regions in the heart (right atrium, left atrium, ventricular apex, aorta, and pulmonary artery) for use in CAT ELISA. We also isolated skeletal muscle (biceps, vastus lateralis, gastrocnemius, soleus, tibialis anterior, masseter, tongue, and diaphragm). Additionally, we initially isolated non-muscle tissue (liver, lung, kidney, spleen, and brain, and ovary) and smooth muscle tissues (stomach, small intestine, uterus, and urinary bladder) from each line. Dissected tissue was immediately frozen in liquid nitrogen. For each time point above, samples from a non-transgenic rabbit were obtained and analyzed for non-specific cross-reactivity in the CAT ELISA.

Proteins for CAT ELISA were obtained by homogenizing the tissues in a small volume (200 -400 mL) of 0.25M Tris (pH 7.8) using a Tekmar homogenizer (Tekmar Company, Cincinnati, OH). The homogenate was incubated at 65°C for ten minutes then centrifuged for ten minutes at 12,000 rpm in a tabletop microfuge. The supernatant was transferred to a new tube and the protein concentration determined.

CAT ELISAs were performed with a microtiter kit according to the manufacturer's instructions. (Boehringer-Mannheim, Indianapolis, IN). A standard curve was performed with each analysis so that test results could be compared between different experiments and production lots. The initial experiments in each line used 50 mg protein samples; this was decreased as needed depending upon the expression level of a given line to ensure that the test results remained within the linear range of the standard curve.

In order to determine the sites of MyHC promoter activity, endogenous expression of α–MyHC and β–MyHC in the rabbit was determined by RNA dot blot. The relative expression of the two MyHC isoforms in rabbit atria and ventricular apex was examined at

expressed only the "fast" α–MyHC isoform at all ages tested, consistent with the published expression pattern of α–MyHC. In the ventricles, over time a progressive decrease in α–MyHC message with a concomitant increase in the "slow" β–MyHC message was observed. A significant level of α–MyHC expression in the mature rabbit ventricle was observed, with the ratio of α–MyHC to β–MyHC in the mature rabbit similar to that of non-diseased adult human heart, suggesting that like the human heart, the mature rabbit heart retains the ability to further shift the ratio of α–MyHC to β–MyHC in response to cardiovascular stress.

EXAMPLE 4

Skeletal muscle expression patterns of α and β MyHC in the transgenic rabbit

The pattern of α–MyHC and β–MyHC expression in a variety of skeletal muscles was examined since rabbit tissues that normally express α–MyHC or β–MyHC are potential sites for mouse promoter activity. RNA dot blots were performed using total RNA extracted from the biceps, vastus lateralis, tibialis anterior, gastrocnemius, soleus, masseter, tongue, and diaphragm of 10 day old, 6 week old, and 16-week-old rabbits. They were hybridized with the α-MyHC, β-MyHC, and GAPDH probes as described in Example 3. Alpha MyHC is strongly expressed in the masseter at 6 weeks and 16 weeks, but not at 10 days. Both α–MyHC and β–MyHC are expressed in the diaphragm, with α–MyHC present at low levels at all three time points and β–MyHC expression increasing with age. As expected, the soleus muscle had β–MyHC expression at all time points, with a very low level of α–MyHC detectable at 10 days but not at 6 weeks or 16 weeks. Beta MyHC expression was demonstrated at very low levels in the biceps and gastrocnemius at all three timpoints assayed.

The results discussed here as well as those from other workers demonstrate that wild type rabbits have significant expression of the fast α–MyHC isoform in the masseter. Also, both fast α–MyHC and the slow β–MyHC isoform expression is seen in the diaphragm, and slow β–MyHC isoform expression in the soleus. These results lead to an interest in determining if the mouse α–MyHC and β–MyHC promoters would be active in the rabbit muscles that normally express these isoforms. Accordingly, CAT ELISA's were performed on multiple skeletal muscle tissues, including the biceps, vastus lateralis, gastrocnemius, tibialis anterior, soleus, tongue, masseter, and diaphragm.

Figure 3 shows the level of CAT expression in the masseter, diaphragm, and soleus as determined by CAT ELISA in lines 286 and 290 (with 2 and 14 diploid copies of the

transgene, respectively) in α-CAT rabbits at 3-5 days, 4-6 weeks, and >16 weeks A: Alpha CAT line 286. B: Alpha CAT line 290. Two non-sex matched rabbits were analyzed at each time point. These three muscles had the highest overall levels of CAT expression with the remaining five skeletal muscle tissues assayed showing generally lower levels of CAT expression. In line 222, there was no detectable expression of CAT in the skeletal muscles tested and is thus not represented in Figure 3.

EXAMPLE 5

CAT expression in smooth muscle and non-muscle tissue

A critical point for the specificity and usefulness of these promoters is that expression be restricted to the desired tissue types, that is, striated muscle. To assess mouse α-MyHC promoter activity in non-striated muscle, CAT-ELISA's were performed on protein extracts from a number of smooth muscle (stomach, small intestine, urinary bladder, and uterus) and non-muscle sites (liver, lung, kidney, spleen, brain, and ovary). These results are summarized in Table 2 and show that the mouse α-MyHC promoter is striated muscle specific in the rabbit.

Table 2: Non-cardiac expression of CAT

Tissue	Line 222 (10 days)	Line 286 (6 weeks)	Line 290 (8 weeks)
RA	19	90	
Ventricular apex	5	32	95
Liver	0	0	1
Lung	0	0	2
Kidney	0	0	0
Spleen	0	0	0
Brain	0	0	0
Stomach	<1	<1	0
Small intestine	0	1	13
Bladder	0	0	0
Uterus	N/A	0	0
Ovary	N/A	0	0

Numbers indicate the picograms of CAT detected per microgram of protein assayed. A complete CAT ELISA series was performed on the first animal tested from each line. N/A = not applicable (male rabbit).

EXAMPLE 6

Homogeneity of CAT expression in tissues

To see whether CAT was expressed homogeneously, CAT in situ immunohistochemistry was performed on papillary muscle from an 8-week-old F2 from the high-expressing line 290 (Figure 2). A section of rabbit papillary muscle was obtained, stained with anti-CAT antibody, and examined under darkfield microscopy. The results are shown in Figure 2 where "A" corresponds to Alpha CAT line 290 papillary muscle and "B" Nontransgenic papillary muscle. CAT was distributed homogeneously throughout the muscle. The staining protocol has been described in detail elsewhere (Knotts S, Sanchez A, Rindt H, et al.: 1996. Developmental maodulation of a beta myosin heavy chain promoter-driven transgene. Dev Dyn 206:182-192), with the anti-CAT-digoxigenin antibody preabsorbed to rabbit heart powder (obtained from acetone precipitation) rather than mouse embryo powder.

The procedure was also modified as outlined below for the use of cryosections rather than paraffin embedded tissue. Papillary muscle tissue was embedded in Tissue-Tek O.C.T. compound (Miles, Inc., Elkhart, IN). Twelve micrometer cryosections were placed on positively-charged slides and the sections allowed to air dry for one hour before fixing with ice-cold acetone for twenty minutes. Excess acetone was blotted away and the slide allowed to air dry. Dehydration and bleaching of the tissue and all subsequent steps were then performed basically as described by Knotts et al. (1996, Dev Dyn, Vol. 206, ppg. 182-192) with a primary antibody concentration of 1:1000, secondary antibody concentration of 1:500, and exposure time of 24 hours.

EXAMPLE 7

Developmental expression in the rabbit transgenic

To analyze mouse promoter activity in transgenic rabbits, the amount of the reporter protein, CAT, was examined by CAT enzyme linked immunoabsorption assay (ELISA). CAT ELISA was chosen over CAT transcript analysis (which may not reflect protein accumulation) or CAT activity assay as a standardized and reproducible method to quantitate the amount of CAT protein.

Of the six α/CAT lines which had transgenic offspring, three lines had CAT expression as assessed by CAT ELISA. The analysis of line 286, the only line to transmit the transgene in a germline pattern of transgene integration (i.e., approximately 50% of each litter born to the F0 was transgenic), was performed on F1 generation rabbits, while the remaining two lines, lines 222 and 290, were analyzed with F2's. All three lines exhibited a different pattern of expression with levels of CAT changing over time.

In contrast to experiments using the mouse α–MyHC promoter in the mouse, the expression patterns and levels was not clearly copy number dependent. The CAT expression patterns and copy number for the three α/CAT lines are shown in Figure 1.

The amount of chloramphenicol acetyl transferase (CAT) was quantitated by ELISA in the left atrium (LA), right atrium (RA), and ventricular apex (APEX) of transgenic rabbits at ages 3-5 days, 4-6 weeks and >16 weeks. Two animals were analyzed at each time point. A. Alpha CAT line 222. B. Alpha CAT line 286. C. Alpha CAT line 290. Note the difference in scale for this line. RA = right atrium, LA = left atrium, APEX = ventricular apex.

In the wild type rabbit, α–MyHC is the only isoform expressed in the atria at any time point. α–MyHC expression is initially high in the ventricle but gradually decreases as the rabbit matures, being replaced by the β–MyHC isoform. None of our three α/CAT lines exactly mimicked the endogenous pattern. Line 222, with 8 diploid copies of the transgene, showed a progressive increase in the amount of CAT present in the atria with age to approximately 300 pg CAT/mg protein seen at the oldest age assayed. There was low and relatively constant expression of CAT in the ventricular apex (Fig 1A). Line 286, with 2 copies of the transgene had very low levels of CAT in the atria at all time points tested and modest and essentially unvarying expression in the ventricular apex (Fig. 1B). Line 290, with 14 copies of the transgene, initially had high levels of CAT in the atria (approximately 800-1000 pg CAT/mg protein) with attenuation of expression over time to almost undetectable levels at 16 weeks (Fig. 1C).

Ventricular expression was extremely high earlier in development, in the order of 3000-7000 pg CAT/mg protein decreasing to 300 - 500 pg CAT/mg protein at 16 weeks. The levels of CAT expression seen in these three lines compare favorably with the levels seen in transgenic mice when the mouse α– and β–MyHC promoters were first characterized and are sufficient to drive transgene expression at a level in which abundant proteins in the heart or other striated muscle tissues can be replaced by transgenically-encoded sequences. These data indicate that the mouse α–MyHC promoter is capable of driving transgene expression at levels necessary to effect complete replacement of a sarcomeric protein with a transgenically-encoded polypeptide.

EXAMPLE 8

Endogenous expression of α-MyHC and β-MyHC in the heart

After observing such high levels of cardiac expression, especially in line 290, the question of whether levels of endogenous α-MyHC and β-MyHC expression were suppressed, presumably from competition for rate limiting factors of gene expression became apparent. Such a non-specific "squelching" or inhibition might significantly limit the general usefulness of the promoters for remodeling heart or skeletal protein complements.

Figure 4 shows an RNA dot blot experiment comparing the expression of α-MyHC and β-MyHC in the right atrium (RA), left atrium (LA), and ventricle (V) in a line 290 heart at 12 weeks. Transgenic (TG) expression is compared to an age-matched nontransgenic rabbit (NTG). No significant difference was found between the TG and NTG animals in endogenous rabbit α-MyHC and β-MyHC expression despite the very high levels of transgene expression in line 290, suggesting that even extremely high levels of transgene expression do not lead to inhibition of endogenous RNA expression.

EXAMPLE 9

Activity of the mouse β-MyHC promoter in the rabbit

As noted above, unlike the mouse, in the rabbit ventricle it is the β-MyHC promoter that is most active. This is also the case in the human ventricle. To determine if the mouse β-MyHC promoter was capable of driving significant levels of transgene expression in the rabbit, the corresponding β/CAT construct was used to generate transgenic rabbits. A similar strategy as for the generation of α-CAT founders was used with the β-MyHC construct. Four founders were obtained and we have analyzed CAT expression in one line (Fig. 5). The data show that expression closely reflects endogenous β-MyHC expression. That is, expression occurs at high levels in the ventricles relative to atrial expression, and also at high levels in the slow muscle types. No significant expression occurred in the non striated muscles or in non muscle tissues.

EXAMPLE 10

The generation of technique involves genetically engineering the α-MyHC and β-MyHC promoters to express the gene of interest producing a construct. Any type of vector could be used, viral, plasmid, or naked DNA. Next the construct is transfected into the cell line using a variety of techniques known by those of skill in the art. If the cell line contains the correct transcription factors, or is related to a striated muscle cell, the gene of interest will be expressed. Analysis of the outcome of expression of the gene is specific to the experimental system.

EXAMPLE 11

This example addresses the use of a gene transfer vector to express the dystrophin, utrophin, dystrophin mini gene, or related genes in a target muscle cell line. A vector is constructed using recombinant techniques to express the gene of interest under control of the murine MyHC promoters. A recombinant construct is then transferred to the animal or human in an appropriate manner. For example, viral vectors can be injected intraveneously, intramuscularly, or subcutaneously. Naked DNA and liposomes will be injected intramuscularly. Vectors or DNA are mixed with an appropriate buffer and solutions supportive to the virus, liposomes, or DNA.

EXAMPLE 12

Muscle-related disease expression vector

A muscle-related disease gene such as dystroglycan (for use in limb-girdle dystrophy), emerin (for use in Erery Dreifuss disease), and tropomyosin (for use in nemaline rod myopathy), is genetically engineered to be expressed by the murine MyHC promoters. A delivery system is chosen, then it is transferred to the animal or human in an appropriate manner. For example, viral vectors can be injected intraveneously, intramuscularly, or subcutaneously. Naked DNA and liposomes will be injected intramuscularly. Vectors or DNA will be mixed with an appropriate buffer and solutions supportive to the virus, liposomes, or DNA.

EXAMPLE 13

A non-muscle-related disease gene such as a gene encoding an antigen or an antisense gene, or Factor IX or decorin, or any other gene of interest, is genetically engineered to be expressed systemically by the murine MyHC promoters. A delivery system is chosen and then used to transferred the gene of interest under MyHC promoter control to the animal or human in an appropriate manner. For example, viral vectors can be injected intraveneously, intramuscularly, or subcutaneously. Naked DNA and liposomes will be injected intramuscularly. Vectors or DNA will be mixed with an appropriate buffer

and solutions supportive to the virus, liposomes, or DNA. The exogenous DNA will be expressed in the muscle and secreted into blood or lymph where it can travel to the therapeutic site.

EXAMPLE 14

Non-murine transgenic animals

Using the described in Example 2, other trans-species transgenics are produced using the murine α-MyHC and β-MyHC promoters. A construct is engineered containing a cardiac-related or therapeutic gene under the control of one of these promoters. The transgenic will be produced following the steps outlined in Example 2. Following introduction of the exogenous gene, cardiac function in these transgenic animals is monitored to determine the effect of the exogenous gene. Monitoring of cardiac function is performed using standard methods known to those of ordinary skill in the art.

EXAMPLE 15

Muscle function in non-murine transgenics

Trans-species transgenics containing genes controlled and regulated by the murine α-MyHC and β-MyHC promoters are constructed using the methods described above. A construct is produced containing a muscle-related gene or antisense under the control of one of these promoters. The promoter construct will be injected into the fertilized egg, zygote, or blastocyst. Following the introduction of the exogenous gene of interest, muscle function in the transgenic animal is observed, and compared to wild type muscle function using standard techniques well known to those of skill in the art.

Conclusion - α-MyHC and β-MyHC promoters show skeletal muscle and cardiac specific expression.

It is clear from the above studies with the rabbit transgenic that the α-MyHC and β-MyHC promoters are differently regulated when used trans-species. The surprising result that trans-species, the promoters are specifically active only in cardiac and skeletal muscle (or striated muscle) can be used in a number of ways. As previously mentioned, there has long been a need for a tissue specific method of gene transfer. Therefore muscle-specific promoters which show a high level of expression are optimal for filling this need. There are a number of uses for such promoters, including gene therapy of a number of diseases, gene transfer *in vitro*, and production of heart and muscle specific transgenics.

WHAT IS CLAIMED IS:

1. A vector for expressing an exogenous DNA in a muscle-specific manner comprising:

a promoter comprising a murine myosin heavy chain promoter or a variant thereof capable of expressing in a muscle-specific manner; and

an exogenous DNA.

2. The vector of Claim 1 wherein the murine myosin heavy chain promoter is the α or β murine myosin heavy chain promoter.

3. The vector of Claim 1 wherein said promoter specifically expresses said exogenous DNA in striated muscle.

4. The vector of Claim 1 wherein said promoter and exogenous DNA are contained in a delivery system.

5. The vector of Claim 3 wherein said delivery system is selected from the group consisting of viruses, plasmids, liposomes, and naked DNA.

6. The vector of Claim 1 wherein said exogenous DNA is selected from the group consisting of muscle-specific genes, heart-specific genes, anti-inflammatory genes, antisense DNA, ribozymes, and systemic disease genes.

7. The vector of Claim 6 wherein said DNA is a muscle-specific gene selected from the group consisting of the Dystrophin gene, the Dystrophin mini-gene, the Utrophin gene, and variants thereof.

8. The vector of Claim 6 wherein said DNA is a muscle-specific gene selected from the group consisting of dystroglycans, emerin, and tropomyosin.

9. The vector of Claim 6 wherein said DNA is a systemic disease genes selected from the group consisting of Factor IX and decorin.

10. A method for expressing exogenous DNA in a muscle-specific manner in a cell or tissue comprising:

a) selecting an exogenous gene;

b) producing a construct which operably links said gene to a promoter comprising a murine myosin heavy chain promoter or a variant thereof capable of expressing in a muscle-specific manner, thereby producing a promoter construct; and

c) delivering said promoter construct to said cell or tissue using a delivery system.

11. The method of Claim 10 wherein said myosin heavy chain promoter is the α or β myosin heavy chain promoter..

12. The method of Claim 10 wherein said said promoter only expresses said exogenous DNA in striated muscle.

13. The method of Claim 10 wherein said exogenous DNA is selected from the group consisting of muscle-specific genes, heart-specific genes, anti-inflammatory genes, antisense DNA, ribozymes, and systemic disease genes.

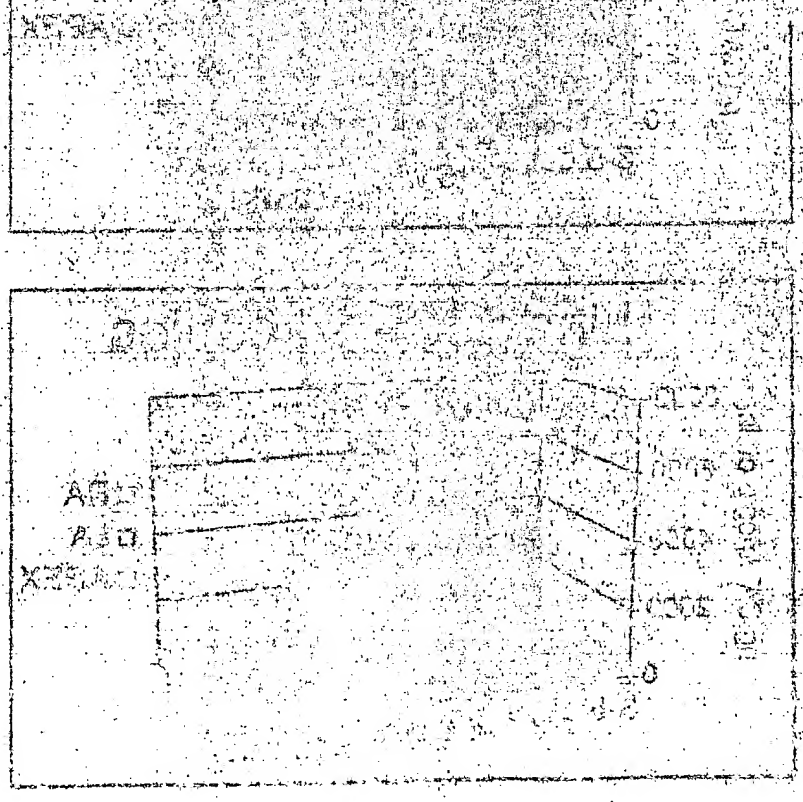
14. The method of Claim 13 wherein said DNA is a muscle-specific gene selected from the group consisting of the Dystrophin gene, the Dystrophin mini-gene, the Utrophin gene, and variants thereof.

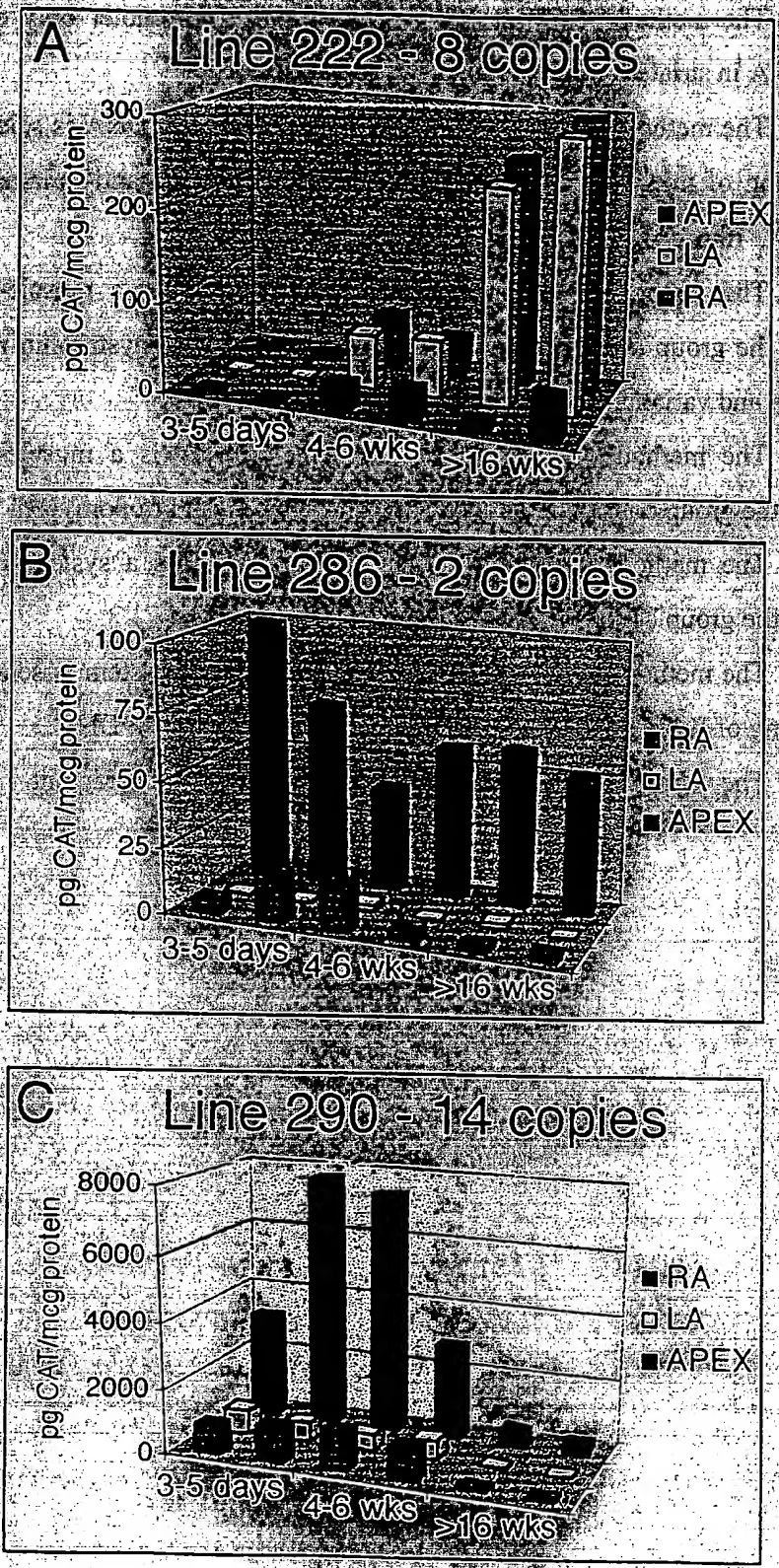
15. The method of Claim 13 wherein said DNA is a muscle-specific gene selected from the group consisting of dystroglycans, emerin, and tropomyosin

16. The method of Claim 13 wherein said DNA is a systemic disease gene selected from the group consisting of Factor IX and decorin.

17. The method of Claim 10 wherein said delivery system is selected from the group consisting of a viral vector, a plasmid, a liposome, and Naked DNA.

18. The vector of any one of Claims 1-9 for use as a medicament.

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200
100
0
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4-6 wks
>16 wks
APEX
LA
RA
B
Line 286 - 2 copies
pg CAT/mcg protein
100
75
50
25
0
3-5 days
4-6 wks
>16 wks
RA
LA
APEX
C
Line 290 - 14 copies
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Fig. 1

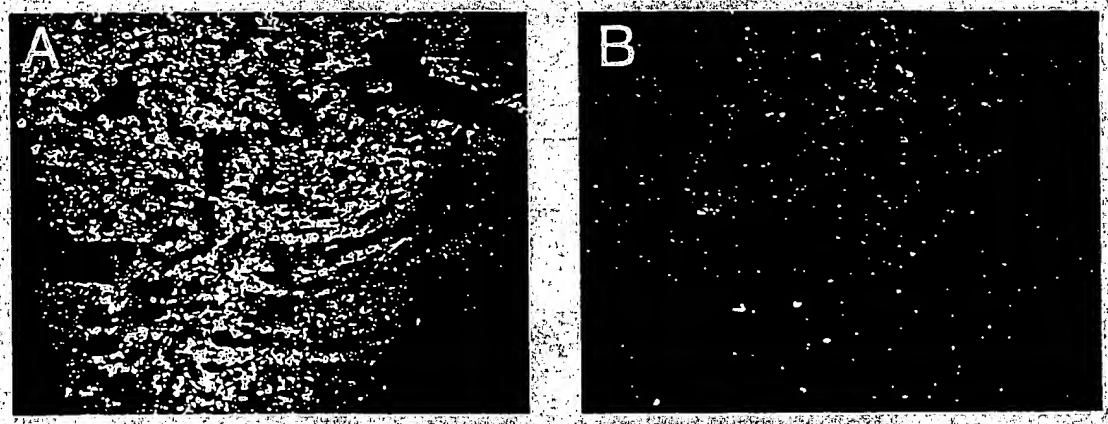
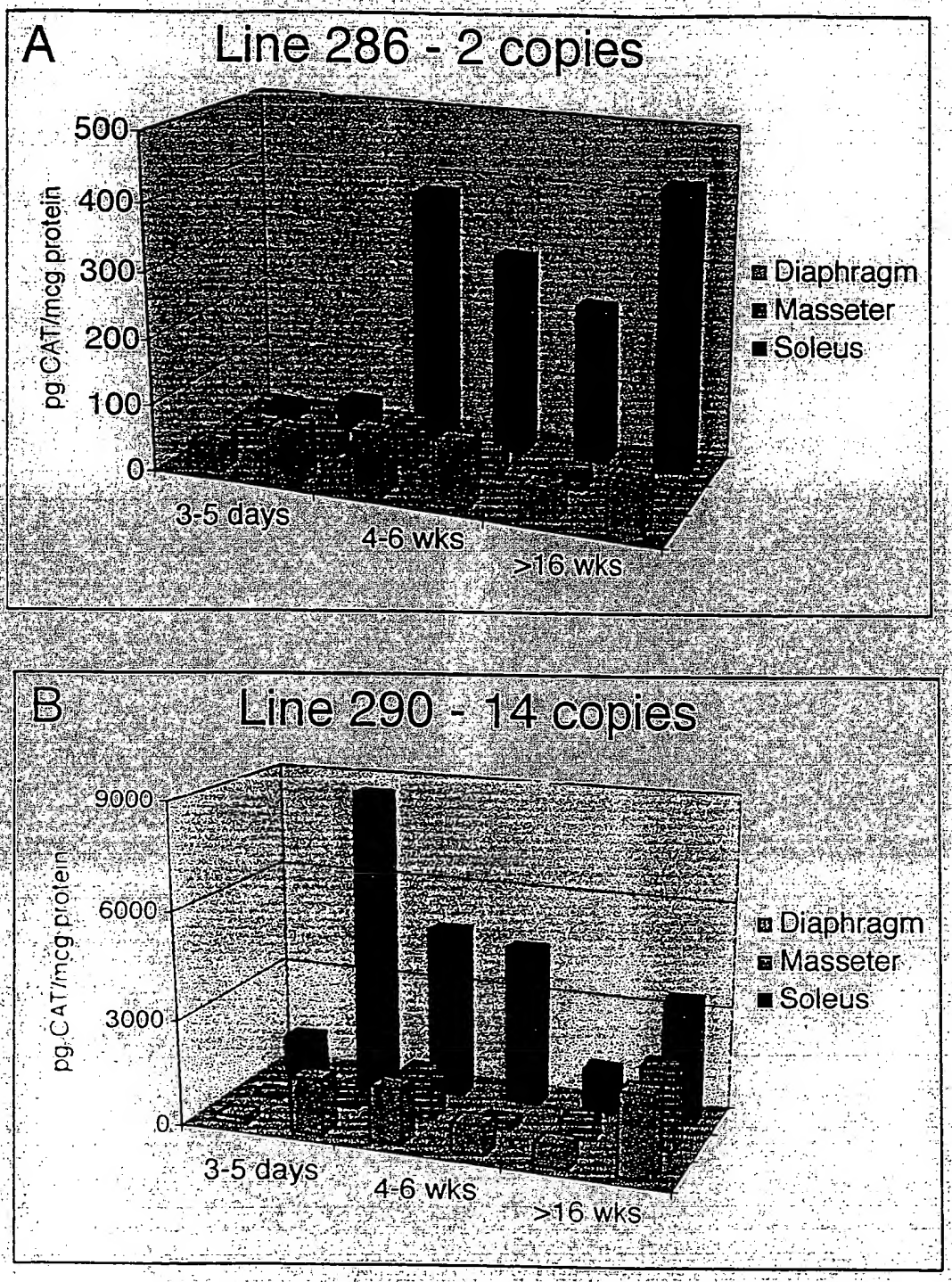


FIG. 2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3-5 days
4-6 wks
>16 wks
Diaphragm
Masseter
Soleus
B
Line 290 - 14 copies
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Masseter
Soleus

Fig 3

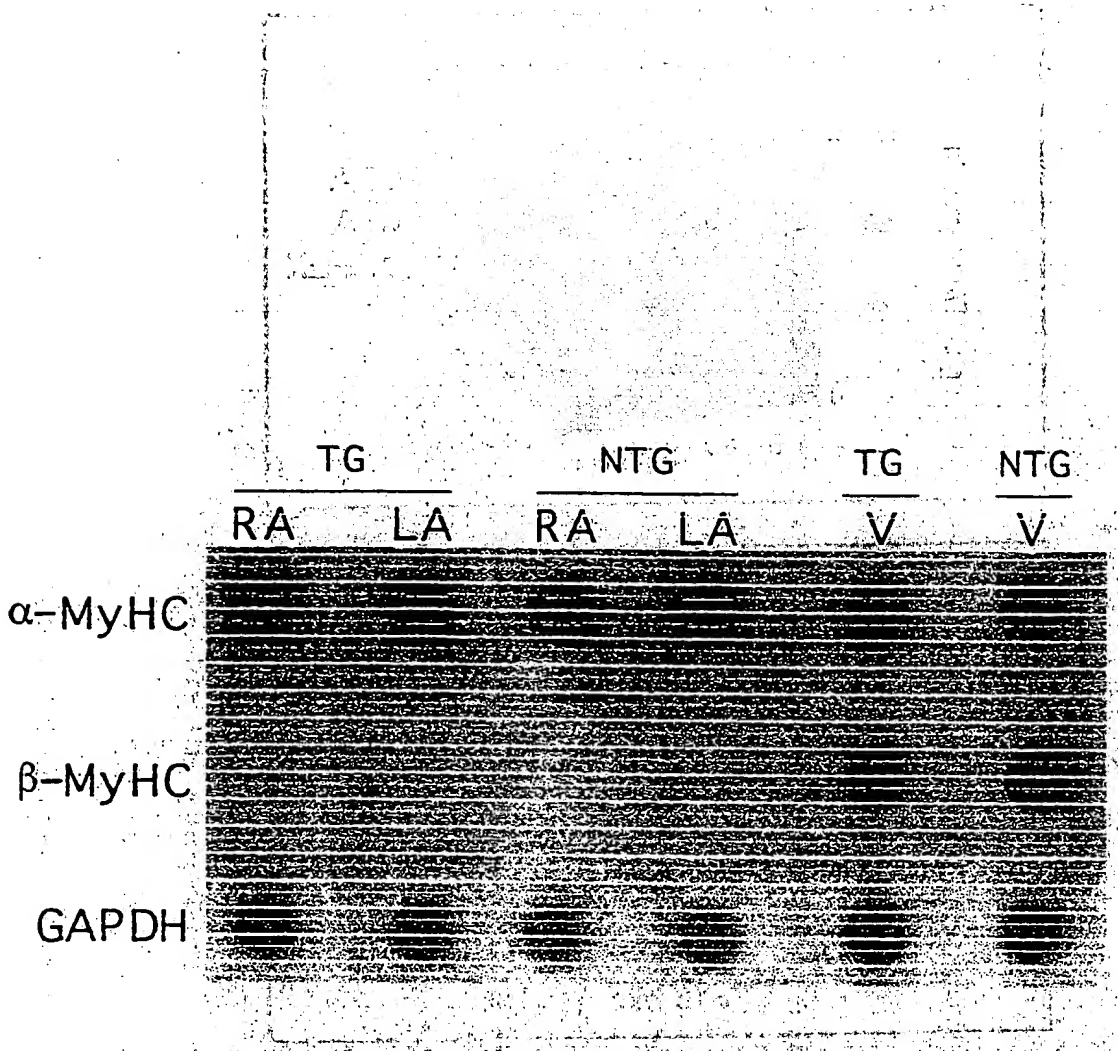
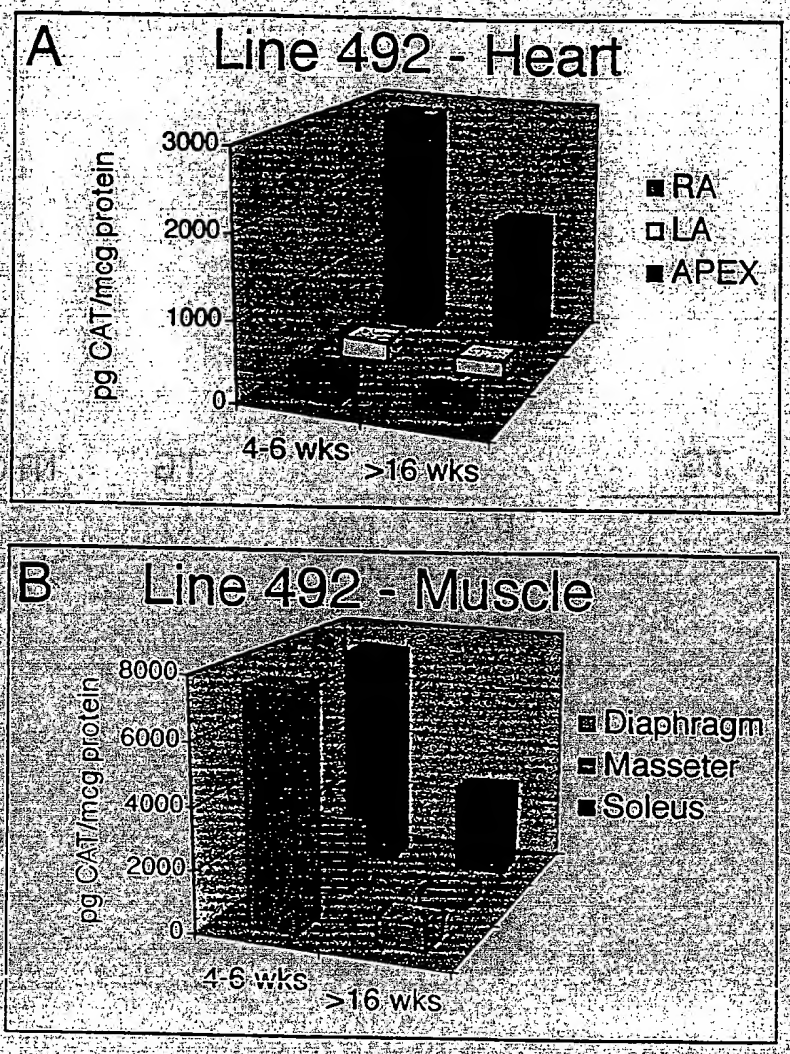


Fig-4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2000
1000
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4-6 wks
>16 wks
RA
LA
APEX
B
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pg CAT/mcg protein
8000
6000
4000
2000
0
4-6 wks
>16 wks
Diaphragm
Masseter
Soleus

Fig 5

INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/US 99/08710

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/85 A01K67/027 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KNOTTS, STEPHANIE ET AL.: "Developmental modulation of a beta myosin heavy chain promoter-driven transgene." DEVELOPMENTAL DYNAMICS, (1996) VOL. 206, NO. 2, PP. 182-192. , XP002110660	1-5, 10-12,17
Y	the whole document	6-9, 13-16,18
X	RINDT, HANSJORG ET AL.: "In vivo analysis of the murine beta - myosin heavy chain gene promoter." JOURNAL OF BIOLOGICAL CHEMISTRY, (1993) VOL. 268, NO. 7, PP. 5332-5338 , XP002110661	1-5, 10-12,17
Y	the whole document	6-9, 13-16,18

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[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 August 1999	12/08/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2	

INTERNATIONAL SEARCH REPORT

Intr ional Application No
PCT/US 99/08710

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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